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Original Article

Voluntary thermal maximum of grassland vipers (*Vipera* spp.): environmental drivers and local adaptation

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ABSTRACT

The thermal tolerance of ectotherms is a critical factor that influences their distribution, physiology, behaviour, and, ultimately, survival. Understanding the factors that shape thermal tolerance in these organisms is, therefore, of great importance for predicting their responses to forecasted climate warming. Here, we investigated the voluntary thermal maximum (VT_{max}) of nine grassland viper taxa and explored the factors that influence this trait. The small size of these vipers and the open landscape they inhabit render them particularly vulnerable to overheating and dehydration. We found that the VT_{max} of grassland vipers is influenced by environmental temperature, precipitation, short-wave flux, and individual body size, rather than by phylogenetic relatedness. Vipers living in colder environments exhibited a higher VT_{max} , contradicting the hypothesis that environmental temperature is positively related to VT_{max} . Our findings emphasize the importance of considering local to regional adaptations and environmental conditions when studying thermal physiology and the evolution of thermal tolerance in ectotherms.

Keywords: thermal physiology; environmental temperature; bioclimate; CHELSA; phylogenetic signal; VT_{max}; *Vipera ursinii*; *Vipera renardi*

INTRODUCTION

Climate change is currently the most pressing global environmental issue, causing a range of effects on all lifeforms (Butchart *et al.* 2010). Several studies predict that some organisms are unable to adapt to the effects of climate change (Hoffmann and Sgrò 2011, Hoffmann *et al.* 2013), which is of particular concern for ectothermic species. These animals rely on environmental heat sources to reach body temperatures for proper physiological functioning and are, therefore, sensitive to climatic shifts in temperature. Thus, environmental temperature is a major constraint on ectotherm distribution and abundance (Sinervo *et al.* 2010, Camacho *et al.* 2018). Understanding the adaptive capability of such species to climate change is vital; however, there are still

gaps in our knowledge in this regard (Aubret and Shine 2009, Sinervo *et al.* 2010).

Reptiles are among the most threatened vertebrates. On a global scale, about 20% of known species are threatened with extinction (Böhm *et al.* 2013). Their populations are declining mainly due to habitat loss, the growing presence of invasive species, environmental pollution, the spread of diseases, and climate change, which affects all these processes (Gibbons *et al.* 2000). Ectothermic populations decline due to climate change, both because of the change in their activity time and the rapid transformation of their communities, trophic networks, and essential habitat properties. Furthermore, reptiles rely on environmental heat for their growth and reproduction, thus the effectiveness of thermoregulation directly affects their fitness (Angilletta 2009).

At the individual level, increasing environmental temperatures due to climate warming can chronically exceed the upper limit of the temperature tolerance of animals (Deutsch *et al.* 2008, Sunday *et al.* 2010, Hoffmann *et al.* 2013). Therefore, there is a dire need for studies on thermal tolerance and behaviour to improve our understanding of the vulnerability of ectothermic species to global warming.

Within a given genus, differences in thermal physiology among species may suggest significant evolutionary flexibility among species (Espinoza *et al.* 2004, Rodríguez-Serrano *et al.* 2009). For instance, comparative studies have shown that related species living in different habitats exhibit variations in body temperature according to the local thermal landscapes (Kingsolver *et al.* 2007, Broitman *et al.* 2009, Rodríguez-Serrano *et al.* 2009, Sinclair *et al.* 2012, 2016). However, the degree to which thermal physiology is influenced by local adaptation versus phylogenetic history is debated and remains an active area of research. While some studies suggest that upper thermal limits, such as critical thermal maximum (CT_{max}) and upper lethal temperature (ULT), are conserved within clades (Grigg and Buckley 2013, Hoffmann *et al.* 2013) and associated with available environmental temperatures (Clusella-Trullas and Chown 2014, Richter-Boix *et al.* 2015), others find no evidence of phylogenetic constraints on these thermal physiology traits (Clusella-Trullas *et al.* 2011).

Understanding the upper limit of thermal tolerance is crucial in studying the thermal physiology of reptiles, as it provides important insights into their capability to regulate their body temperature in response to environmental conditions (Virens and Cree 2019). For reptiles, CT_{max} and voluntary thermal maximum (VT_{max}) are commonly used parameters to assess different aspects of thermal tolerance (Camacho and Rusch 2017). In a heating setup, when the body temperature reaches VT_{max} , the animal attempts to escape high temperatures by moving to a cooler microhabitat (Camacho and Rusch 2017). If this is impossible, the reptile's body temperature may reach CT_{max} , then ULT, leading to disorders of movement coordination, muscle cramps, loss of consciousness, and even death (Hutchison 1961, Lutterschmidt and Hutchison 1997). Since the measurement of CT_{max} is hazardous due to the high risk of mortality during measurement, the method of VT_{max} was recommended instead (Camacho *et al.* 2018). However, it is important to note that VT_{max} , CT_{max} , and ULT measure different aspects of thermal tolerance. While VT_{max} reflects behavioural adaptations to avoid heat stress, CT_{max} and ULT indicate physiological limits beyond which serious or lethal effects occur. This underlines the need for a comprehensive approach that considers both behavioural and physiological aspects of thermal tolerance when studying the thermal physiology of reptiles. The measurement of VT_{max} is considered to be a non-damaging and ecologically important measurement in the understanding of the vulnerability of a species to warming climates (Taylor *et al.* 2020). Short-term captivity does not appear to affect VT_{max} , which showed high repeatability, and the habituation of individuals to VT_{max} experiments has not yet been detected in snakes (Díaz-Ricaurte and Serrano 2020).

Here we present VT_{max} measurements and estimates for nine grassland viper species and subspecies of conservation concern. We assess the effects of phylogenetic constraints and local climatic conditions on VT_{max} by testing for the presence of phylogenetic signal in VT_{max} , and modelling the effect of current spatial

variation of the thermal landscape on VT_{max} . We hypothesize that populations living in warmer environments (i.e. lowlands) have higher thermal tolerance compared to alpine taxa, thus we compare VT_{max} among lowland and mountain-dwelling populations. Based on the local climatic conditions, we predict the VT_{max} of populations not directly measured in this study, because many populations of these vulnerable species occur in remote areas and data deficiency hinders the evaluation of the potential impact of threats like climate change. Finally, we also test the effects of individual traits such as sex and body size on VT_{max} , as we expect that larger individuals will show higher thermal tolerance.

MATERIALS AND METHODS

Study taxa

The *Acridophaga* subgenus (hereafter referred to as grassland vipers), encompassing the *Vipera ursinii* complex, *V. renardi* complex, *V. dinniki*, *V. graeca*, *V. walser*, *V. kaznakovi*, *V. darevskii* complex, and *V. anatolica*, constitutes numerous phylogenetically distinct species and subspecies. These are recognized as evolutionary significant units (ESUs) due to the distinct levels of divergence and evident allopatric speciation patterns (Nilson and André 2001; Ferchaud *et al.* 2013; Zinenko *et al.* 2015, Mizsei *et al.* 2017; Freitas *et al.* 2020; Vörös *et al.* 2022). Grassland vipers occupy a wide range of the Palaearctic steppe biome at different elevations, but some taxa also inhabit humid alpine grasslands above the tree line in the Mediterranean and Central Asian mountain chains (Fig. 1). However, this study excluded *V. kaznakovi*, which mostly inhabits closed, subtropical, and deciduous forest habitats (Mebert *et al.* 2015), due to its uncertain phylogenetic position, unknown thermal behaviour, and distinct habitat preferences.

Grassland vipers often live in small, isolated populations, which make them sensitive to habitat fragmentation and degradation (Mizsei *et al.* 2018). Although certain ESUs of grassland vipers are not considered threatened, as their IUCN Red List status is Least Concern, the majority of these taxa face varying levels of extinction risk, ranging from vulnerable to critically endangered (Edgar and Bird, 2006; Maritz *et al.* 2016; Mebert *et al.* 2016, Göçmen *et al.* 2017, Mizsei *et al.* 2018). In light of the current predictions of climate change outcomes, cold-climate-adapted Mediterranean reptiles, including *V. ursinii*, *V. walser*, and *V. graeca*, are the most threatened snakes in Europe (Araújo *et al.* 2006, Carvalho *et al.* 2010, Martínez-Freiria 2015, Mizsei *et al.* 2020).

Data collection

To collect VT_{max} data of grassland vipers we measured wild individuals from representative populations of nine taxa (Fig. 1; Table 1.). We sampled one representative population per taxa, except for *V. graeca* and *V. ursinii* ssp. Cr, where two populations were sampled. We recorded the GPS coordinates of observed individuals. Snakes were caught using gloves and then transported to our field camp, where measurements were taken. Before and after the VT_{max} measurements, each individual was kept in a linen bag in the shade. VT_{max} measurements were followed by sexing the individuals and measuring the snout-vent length (SVL). After measurements, snakes were released where they had been found. We were able to measure VT_{max} of 194 viper individuals of nine taxa (Table 1).

Table 1. SVL and sample size of individuals measured for VT_{max}

	SVL mm mean ± SE (sample size)	
	male	female
<i>Vipera ursinii rakosiensis</i>	228 ± 38.11 (N = 5)	310 ± 24.73 (N = 14)
<i>Vipera ursinii moldavica</i>	307.24 ± 29.07 (N = 17)	344.83 ± 30.78 (N = 12)
<i>Vipera ursinii macrops</i>	209.43 ± 35.92 (N = 7)	256.13 ± 20.7 (N = 15)
<i>Vipera ursinii</i> ssp. Cr	261.33 ± 22.2 (N = 6)	336 ± 20.43 (N = 5)
<i>Vipera dinniki</i>	350 ± 21.84 (N = 6)	401.33 ± 21.78 (N = 9)
<i>Vipera renardi eriwanensis</i>	299.11 ± 10.04 (N = 9)	306.7 ± 14.38 (N = 10)
<i>Vipera renardi renardi</i>	306.77 ± 32.5 (N = 13)	394.1 ± 31.04 (N = 21)
<i>Vipera graeca</i>	241.5 ± 30.63 (N = 4)	294 ± 19.22 (N = 17)
<i>Vipera darevskii</i>	334.4 ± 25.3 (N = 5)	327.79 ± 32.83 (N = 14)

To measure the VT_{max} of grassland viper individuals in the field, we used a portable climate chamber. The climate chamber was a 30 × 30 × 30 cm styrofoam box with an internal volume of 22 × 22 × 22 cm. To enable animals to escape high temperatures, an exit with a circular cross-section (∅ 4 cm) closed by a hinged door was built on the side of the chamber. The internal air temperature was controlled by a precision temperature control unit (Omron E5CC-T), which monitored the air temperature via a PT100 temperature sensor (HVAC-Controls CHWTS-1) and switched a 60 W heating unit (Schrack IUK08343) on or off via a relay (Omron G3NA205B). To ensure a cautious, gradual increase in temperature, a multi-breakpoint heating profile was programmed into the thermal control unit with the CX-Thermo EST2-2C-MV4 v.4.0 software (Omron). At the start of the measurement, the temperature of the box was kept at 25°C for 10 min and then it was automatically increased by 0.5°C every minute. When individuals left the climate chamber, their body temperature (=VT_{max}) was immediately recorded using a sterilized and lubed cloaca thermometer (Testo 826-T4). Each individual was kept in captivity for 2 to 5 days, and their VT_{max} was measured three times during this period.

To estimate climatic conditions on the habitats of the studied taxa, we compiled a database of grassland viper occurrence records based on available databases (GBIF, iNaturalist, UkrBIN), published (Console *et al.* 2020; Mebert *et al.* 2023) and unpublished datasets (<https://openbiomaps.org/projects/vipera>), georeferenced locations from literature (Nilson and Andrén 2001, Ferchaud *et al.* 2012; Zinenko *et al.* 2012), and datasets of the authors (Fig. 1). We validated the records based on the available information for each specimen (morphology) or the observation (photographs). In case of the lack of visual information, we checked the indicated location using satellite imagery and the expected distribution ranges estimated based on molecular data (Ferchaud *et al.* 2012, Zinenko *et al.* 2015, Mizsei *et al.* 2017; Freitas *et al.* 2020; Vörös *et al.* 2022). Dataset compilation and data validation resulted in N = 4266 occurrence coordinates.

To assess the influence of the thermal landscape on VT_{max}, we obtained environmental data from the CHELSA 2.1 database (Karger *et al.* 2017, 2021), which provides data of down-scaled model output temperature and precipitation estimates at a horizontal resolution of 30 arcsecs (approximately 1 × 1 km grid size), based on measurements of atmospheric precipitation

and temperature. The precipitation algorithm used by Karger *et al.* (2017, 2021) incorporates orographic predictors, including wind fields, valley exposition, and boundary layer height, with a subsequent bias correction. The resulting data consist of monthly temperature and precipitation layers, and various derived variables. We used the dataset for recent climate conditions based on data observed between 1981 and 2010.

Data analysis

All analyses were conducted in R 4.1.3 (R Core Team 2022). To estimate VT_{max} for grassland vipers we selected the highest VT_{max} value of each individual. To estimate the distribution of VT_{max} for each taxon, first we calculated the mean, variance, skewness, and kurtosis of the data using the descdist function of FITDISTRPLUS package (Delignette-Muller and Dutang 2015). Second, using these parameters we fitted a distribution function with the rpearson function of the PearsonDS package (Becker and Klößner 2022). Finally, we selected the peak value of the fitted distribution as the estimated mean VT_{max} value of each taxon (eVT_{max}).

To test for the presence of phylogenetic signal in the VT_{max} of the measured taxon, we used the multilocus dated Bayesian phylogenetic tree reconstruction of Freitas *et al.* (2020), which was based on the concatenated dataset of seven mitochondrial gene fragments of all Eurasian vipers. To import and prepare the nexus file of that phylogenetic reconstruction for the subsequent analysis, we applied the APE and PHYTOOLS packages (Revell 2012, Paradis and Schliep 2019). To calculate branch lengths we used the compute.brlen function of APE. We tested for the presence of phylogenetic signal by computing Pagel's lambda (λ; Pagel 1999, Freckleton *et al.* 2002) and Bloomberg's K (Blomberg *et al.* 2007) on eVT_{max} using the phylosig function of PHYTOOLS.

To compare the VT_{max} of lowland and mountain-dwelling grassland viper populations, we created two subsets of the sampled nine taxa based on elevation. Populations on steppes below the treeline (usually 1000 m below sea level) were considered to be steppe populations, whereas those inhabiting alpine grasslands above the treeline in high-mountain environments (usually above 1000 m a.s.l.) were classified as alpine populations. Forest treeline is an ecological boundary that can serve as a barrier among lowland and mountain species in case of geographic



Figure 1. Distribution records of grassland vipers used in the study (white dots) and approximate distribution of their range (polygons) according to the taxonomy in Freitas *et al.* (2020). Numbered points and taxon names in yellow letters indicate populations measured for VT_{\max} and white dots indicate locations used to extract environmental data and estimate pVT_{\max} . Photos by E. Mizsei.

proximity (Hofgaard and Willmann 2002, Hertel *et al.* 2008). The difference of VT_{\max} among these groups was tested by two-sample Wilcoxon tests (also known as Mann–Whitney test) of the `wilcox.test` function of STATS package (R Core Team 2022).

To model the influence of spatial variation of the thermal landscape on VT_{\max} , we used the random forests' machine learning method using the `randomForest` function of the `randomForest` package (Liaw and Wiener 2002) with the default parameters. The CHELSA environmental data were extracted to the coordinates of sampled viper individuals by the `extract` function of the `RASTER` package (Hijmans *et al.* 2020). Extracted data were centred to zero and scaled to a standard deviation of one using the `scale` function. Highly correlated ($r > 0.8$) variables were removed using the `findCorrelation` function of the `CARET` package (Kuhn, 2022). The importance of environmental predictors of VT_{\max} in the random forest model was measured by the mean decrease accuracy (%IncMSE) and by the mean decrease of Gini-index (IncNodePurity), which were calculated using the `varImp` function of the `CARET` package. To predict VT_{\max} (pVT_{\max}) for non-sampled grassland viper taxa, we used the `predict` function of the `randomForest` package, using environmental data at their distribution points of the occurrence dataset (Fig. 1).

To investigate the effect of individual traits on VT_{\max} , we used Markov chain Monte Carlo multivariate generalized linear

mixed models (MCMCglmm). As the dependent variable, we included the VT_{\max} measurement of each individual, and SVL, sex, and their two-way interaction as explanatory variables. Applying phylogenetic control using the previously constructed phylogenetic tree, we fitted the model using the `MCMCglmm` function and the `MCMCglmm` package using uninformative priors (Hadfield 2010). The model was run with up to 5 000 000 MCMC iterations. MCMC parameters were sampled after 1000 iterations (burnin) and every 500 iterations (thin).

RESULTS

Across the sampled nine grassland viper taxa, individual VT_{\max} ranged between 32.5 and 39.7°C, with a mean of 36.4°C (± 0.105 SE, $N = 194$). We observed a wider range of VT_{\max} in *V. ursinii macrops* and *V. ursinii ssp. Cr*, and a narrower range in *V. dinniki* and *V. renardi eriwanensis* (Fig. 2). The phylogenetic position of grassland vipers did not constrain VT_{\max} as we found no evidence of a significant phylogenetic signal in VT_{\max} of the studied viper taxa ($\kappa = 0.873$, $P = 0.296$; $\lambda < 0.0001$, $P = 1$).

Comparison of VT_{\max} between altitudes indicated that lowland taxa (*V. renardi renardi*, *V. ursinii moldavica*, and *V. u. rakosiensis*) had significantly lower VT_{\max} than mountain taxa (*V. graeca*, *V. darevskii*, *V. dinniki*, *V. renardi eriwanensis*, *V. ursinii ssp. Cr*, and *V. u. macrops*; $W = 2113$, $P < 0.0001$).

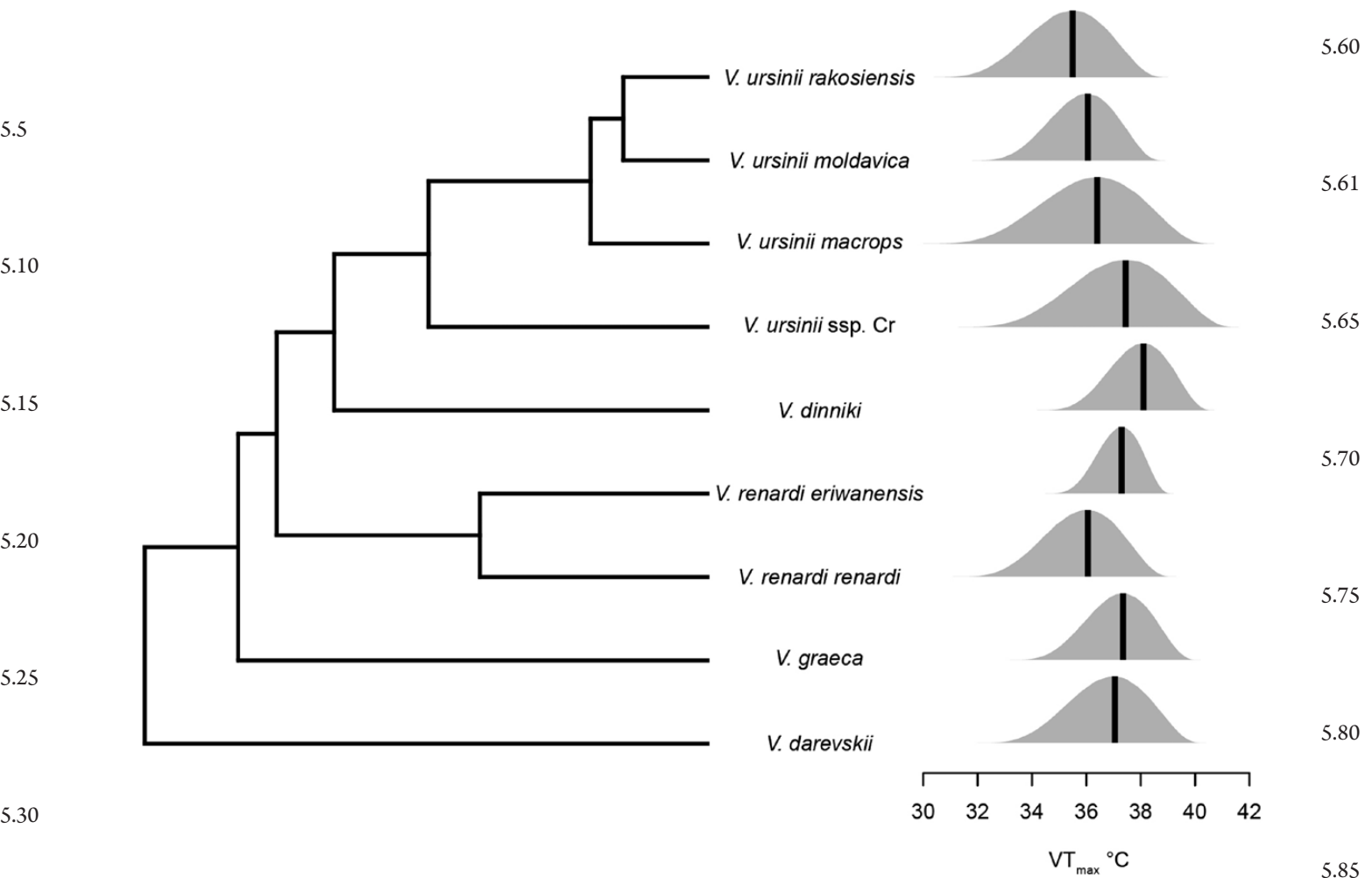


Figure 2. Phylogenetic relationship of the studied taxa and the corresponding distribution of VT_{max} . Black vertical lines indicate peak value (eVT_{max}).

The most important environmental variables to explain variation in VT_{max} were precipitation in the coldest (bio19), driest (bio14), and warmest (bio18) quarters, shortwave flux (rsds), and mean temperature of the coldest (bio11) and wettest (bio8) quarters (Table 2). The model accurately predicted the observed mean of VT_{max} (Fig. 4). The model-averaged coefficient estimates of the GLM indicated that rsds had a significant positive effect on VT_{max} . Meanwhile, bio8, cloud cover (clt), mean temperature range (bio2), and mean temperature of the driest quarter (bio9) had a significant negative effect on VT_{max} (Table 2).

At the individual level, the influence of SVL on VT_{max} was significantly positive, while sex and the interaction of both variables had no effect (Table 3).

DISCUSSION

We found no evidence of a significant phylogenetic signal on the VT_{max} of grassland vipers, indicating that the VT_{max} of these vipers has little phylogenetic inertia. This result is surprising because this is not always the case with other thermal traits. Studies focusing on CT_{max} showed that the evolution of upper thermal limits can be slow and often exhibits strong phylogenetic inertia, a concept often associated with the Bogert effect (Araújo et al. 2013, Grigg

and Buckley 2013). Bogert proposed that regulatory behaviours, such as thermoregulation, shield organisms from selection and potentially limit physiological evolution. This concept, sometimes referred to as behavioural inertia, suggests that behavioural thermoregulation can dampen evolutionary pressures on physiological traits such as CT_{max} (Muñoz 2022). Given that changes in VT_{max} could potentially influence the evolutionary conservatism of CT_{max} , further research is needed to fully understand the different implications of these two measures of thermal tolerance.

On the other hand, several macroevolutionary studies showed that different features of the thermal performance curve can evolve independently from each other (Bodensteiner et al. 2021). While some traits may evolve in a phylogenetically constrained manner, the observed pattern of thermal tolerance in our study might be a consequence of multiple independent adaptations to the local environment. Upper behavioural thermal tolerance like VT_{max} might be influenced by thermal plasticity, which seems to be more likely to change in alpine environments where yearly and daily occurrences of optimal environmental temperatures are limited. This question can be further addressed in the future by collecting data from the *V. kaznakovi* complex, which live in temperate and subtropical forests, and expanding the sampling to the lowland populations of *V. dinniki* and *V. darevskii*.

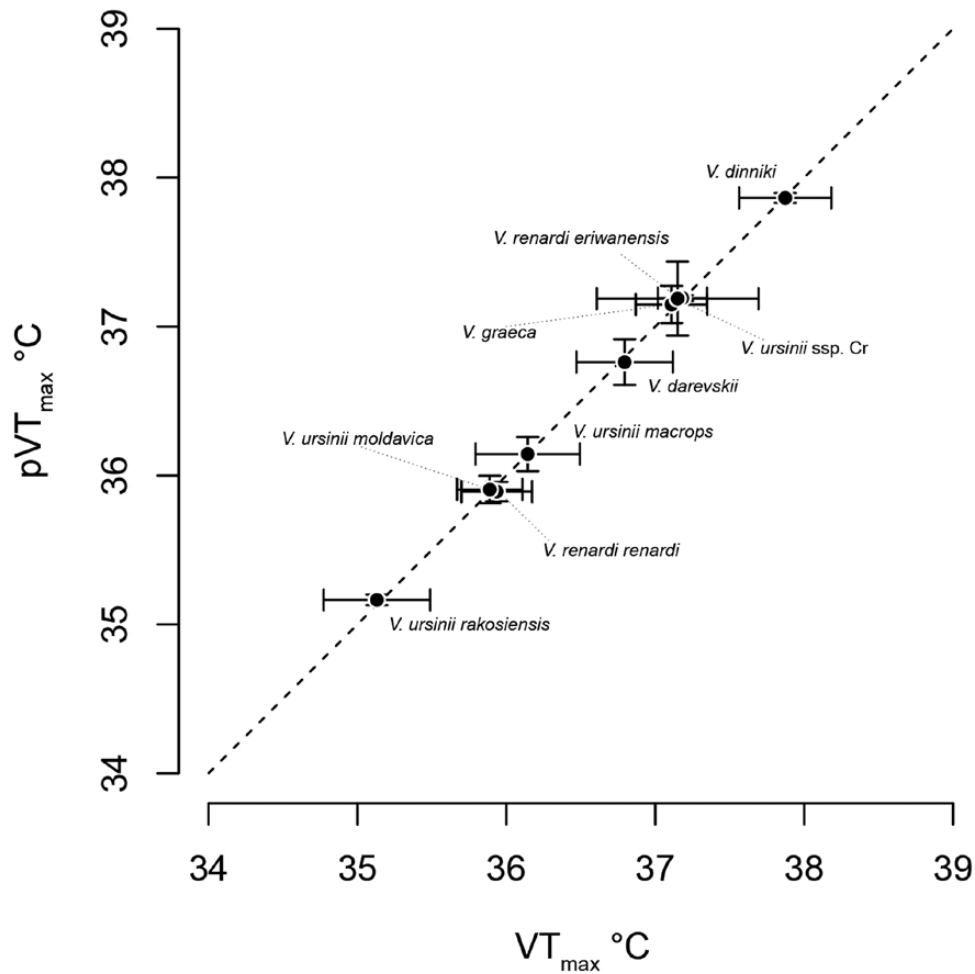


Figure 3. Observed (VT_{max}) and predicted (pVT_{max}) upper thermal tolerance of grassland vipers (mean \pm SE). The pVT_{max} is the prediction of the random forest model fitted using environmental variables. Error bars show SE and the dashed line indicates 1:1 line.

Our study found higher VT_{max} in populations occurring in colder environments, which contradicts the hypothesis that environmental temperature is positively related to VT_{max} . This may be explained by the fact that VT_{max} is higher when temperatures are low during the active season of the vipers when surface humidity and cloud cover are low, precipitation is unevenly distributed during the year, and primary productivity is high. These factors may create a thermal environment that promotes higher thermal tolerance in these vipers, which enables them to more efficiently utilize the short daily moments of sunny weather for physiological purposes, such as digestion and gestation, because otherwise environmental temperatures decrease rapidly at high elevations unlike in lower elevations. The most widely used measurement for thermal tolerance, CT_{max} , is often better predicted by variables that reflect temperature variation than mean temperature variables in all squamates (Clusella-Trullas et al. 2011). However, the cold climate hypothesis suggests that in cold and/or unpredictable climates (Tinkle and Gibbons 1977), there is a strong selection for higher thermal tolerance, which may be reflected in increased CT_{max} and lower CT_{min} in viviparous species, like vipers, leading to higher fitness, contrary to egg-laying species, which cannot control incubation temperature (Shine 1995, Lourdais et al. 2004). In summary, our findings suggest that

high thermal tolerance in cold habitats may be an adaptation to maximize thermoregulation when the environmental temperature exceeds the preferred temperature for short periods only. Further research on the relationship between environmental factors and thermal tolerance in other species, including studies of snake operative and body temperatures in their natural environments, may help to better understand the adaptive significance of thermal tolerance in ectotherms.

The use of macro- and mesoclimate layers has become a standard tool in ecological studies despite the fact that smaller ectotherms have the ability to thermoregulate behaviourally using microsites. These data layers allow us to comprehend the overarching thermal landscape, a critical facet when considering the potential repercussions of substantial climate variations (Kearney and Porter 2009). Fine-scale behaviour, such as microhabitat use and thermoregulatory actions, e.g. basking behaviours, could permit snakes at higher elevations to attain superior body temperatures despite the cooler ambient environment (Huey and Slatkin 1976). Despite these potential divergences, we underscore that ambient and operative temperatures often display a significant positive correlation (Blouin-Demers and Weatherhead 2001), especially in open habitats like those inhabited by our grassland vipers. Nonetheless, despite the

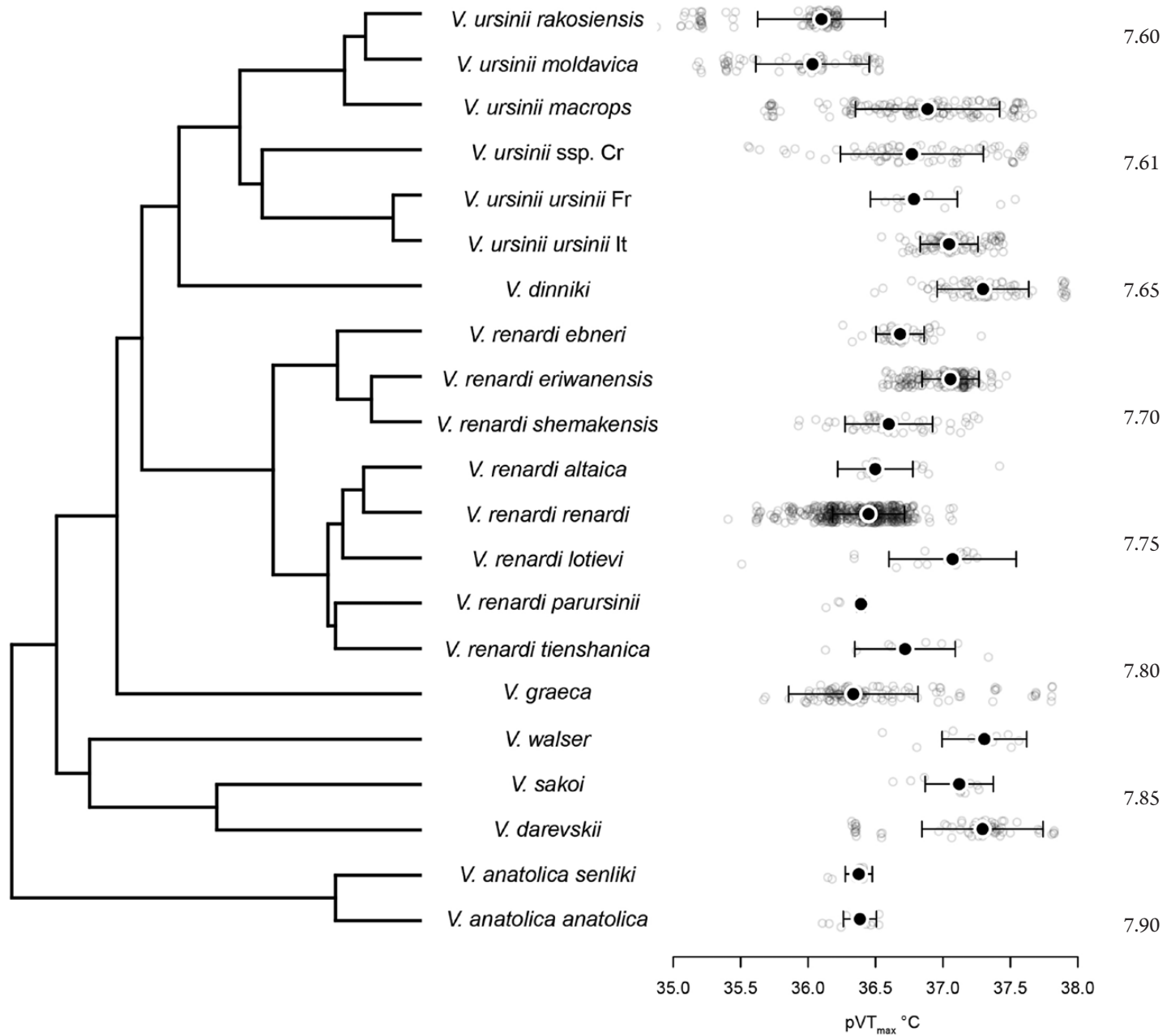


Figure 4. Mitochondrial phylogeny of grassland vipers their predicted (pVT_{max}) upper thermal tolerance. Pale circles indicate pVT_{max} at presence localities and large dots and error bars show mean \pm SE of pVT_{max} .

potential discrepancies among macro-, meso-, and microclimates, the use of climate layers provides an informative and relevant framework for ecological and conservation studies, including climate change research (Elith and Leathwick 2009). Use of data like CHELSA and BIOCLIM provides a comprehensive, large-scale perspective that captures overall trends and patterns that smaller scale studies, focusing solely on operative temperature, might overlook (Kearney and Porter 2009). Thus, while acknowledging these inherent limitations, we believe that the information garnered from broad-scale climate layers, when combined with a thorough understanding of the species' biology and behaviour, can significantly contribute to the formulation of effective conservation strategies in a rapidly changing climate.

While our study provides insights into the thermal tolerance of nine grassland viper taxa, the results should be interpreted with caution due to the relatively small sample size ($N = 194$) and the limited number of taxa studied ($N = 9$). We acknowledge that our study only measured one population per taxon (except *V. graeca* and *V. ursinii ssp. Cr*) and that intra-lineage variation in temperature tolerance among populations remains unknown. To improve the generalization of our results, future studies should consider more populations and select locations that represent the full thermal range of each lineage. While our model predicted VT_{max} values for all locations, we observed lower predicted VT_{max} values for alpine lineages and higher predicted VT_{max} values for lowland lineages. These differences are

Table 2. The most important environmental variables to explain variation in VT_{max} of grassland vipers identified by the random forest and the GLM analyses. Predictor importance in the random forest was measured by mean decrease accuracy (%IncMSE) and by the mean decrease of Gini-index (IncNodePurity), while for the GLM analyses we show the averaged coefficient estimates of the best GLM models ($\Delta AICc < 2$)

Variable	Random forest		GLM				Variable description
	%IncMSE	IncNodePurity	Estimate	SE	Z	P	
bio19	14.17	19.1	1.8728	3.2307	0.5783	0.5630	Precipitation of coldest quarter
bio14	13.57	16.43	-7.0225	4.2226	1.6589	0.0971	Precipitation of driest month
bio18	13.51	14.12	3.7950	2.3127	1.6372	0.1016	Precipitation of warmest quarter
rsds	13.06	20.93	0.8918	0.3018	2.9392	0.0033	Surface downwelling shortwave flux
bio11	12.56	21.3	-5.4016	3.5749	1.5092	0.1313	Mean temperature of coldest quarter
bio8	10.98	21.07	-5.3581	1.8214	2.9267	0.0034	Mean temperature of wettest quarter
bio15	10.91	12.38	-2.3058	2.0478	1.1241	0.2610	Precipitation Seasonality
npp	10.7	10.97	1.3883	1.5382	0.9013	0.3674	Net primary productivity
clt	10.67	10.43	-1.2482	0.5563	2.2367	0.0253	Average monthly total cloud cover
bio2	9.86	7.45	-4.7240	2.0143	2.3374	0.0194	Mean diurnal temperature range
bio9	8.7	6.69	4.7262	1.7328	2.7138	0.0067	Mean temperature of driest quarter
hurs	6.92	9.1	-0.3517	0.9806	0.3574	0.7208	Near-surface relative humidity
bio7	5.11	5.59	6.2093	3.2170	1.9254	0.0542	Temperature annual range

Table 3. MCMCglmm coefficient estimates on the individual level variation of VT_{max}

(Intercept)	Estimate	CI1-95%	CIU-95%	Effective sample	pMCMC
	34.1474	31.9693	36.3887	928.6708	0.001
SVL	0.0108	0.0051	0.0177	990.5308	0.001
sex	0.6837	-0.4969	1.7835	972.1719	0.2265
SVL:sex	-0.0036	-0.0074	-0.0004	979.921	0.0501

explained by the geographical variation of the thermal landscape, i.e. for all taxa we can find colder or warmer habitats also, than the sampled one. Overall, our study provides a starting point for future investigations into the thermal tolerance of grassland vipers and highlights the need for a more comprehensive understanding of the environmental factors that shape thermal tolerance in ectotherms.

Studies on thermal physiology are particularly important to estimate the viability of ectotherms in the face of unprecedented habitat loss during the last decades and the rapidly changing climate. According to Nowakowski *et al.* (2018), species with low upper thermal limits are more vulnerable to habitat modification, because these activities alter local microclimates, along with community-level changes. Interestingly, the taxa showing the lowest VT_{max} , *V. u. rakosiensis* and *V. u. moldavica*, are close to the brink of extinction due to extremely high degrees of habitat loss and habitat degradation (Mizsei *et al.* 2018). We suppose that the current conservation status of these taxa is not a consequence of low upper thermal limits; however, conservation activities should take into consideration the often ignored thermal biology traits in case of restoration of critically endangered populations as thermal specialists are expected to perform better than generalists under a narrow range of temperatures (Clavel *et al.* 2011) but are more sensitive to changes in the thermal landscape (Nowakowski *et al.* 2017).

Habitat degradation and/or change in the thermal landscape can cause acute and chronic stress (Brischoux *et al.* 2016,

Josserand *et al.* 2017). Acute or chronic thermal stress can directly reduce the fitness of individuals in habitats where maximum daily temperatures, including those in daytime refugia, regularly approach upper thermal limits (Rittenhouse *et al.* 2008, Nowakowski *et al.* 2015). Although diurnal reptiles can reduce their activity to avoid overheating, this can still result in limited time spent on foraging and reproduction, which may ultimately lead to lower fitness and population declines. However, it is important to note that vipers, and possibly other species, can switch to nocturnal activity as a highly adaptive behavioural response to warming temperatures, at least in lower elevations, thus, conserving energy by waiting in safe shelters during the day (Pough, 1983). Thus, conservation efforts aimed at mitigating the effects of climate change may be more effective in the long term than interventions aimed at assisting biodiversity to adapt to the altered thermal landscape either through relocation or other methods (Sinervo *et al.* 2010, Nowakowski *et al.* 2018).

CONCLUSION

In conclusion, our study found that the VT_{max} of grassland vipers is influenced by environmental temperature, precipitation, and individual body size, rather than phylogenetic relatedness. We also found that vipers living in colder environments have higher VT_{max} than vipers living in hotter environments. These results emphasize the significance of considering local adaptation and environmental conditions in the study of thermal physiology

and the evolution of thermal tolerance in ectotherms. By identifying the environmental variables that contribute to the evolution of thermal sensitivity in grassland vipers, our study can help to set methodological standards for similar studies in other ectothermic species. Our study provides a valuable baseline for future investigations that aim to understand general patterns of thermal tolerance in ectotherms, particularly in the face of ongoing habitat loss and climate change.

SUPPLEMENTARY DATA

Supplementary data are available at *Zoological Journal of the Linnean Society* online.

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AUTHORS’ CONTRIBUTIONS

Conceptualization—D.R., E.M., S.L.; methodology—D.R., T.S., E.M.; formal analysis—D.R., E.M.; investigation—D.R., T.S., K.M., B.Ü., M.B., G.R., M.S., S.L., E.M.; resources—D.R., B.Ü., S.L., E.M.; data curation—D.R., E.M.; writing—original draft—D.R., E.M.; writing—review and editing—D.R., T.S., K.M., Ü.B., M.B., G.R., M.S., S.L., E.M.; visualization—E.M.; supervision—S.L., E.M.; funding acquisition—S.L..

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY

Codes for statistical analysis and the data included in the study are archived at the Zenodo repository (10.5281/zenodo.7775746).

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